PROTOCOL TO COLD SHOCK EFFECT IN GFP EXPRESSION COORDINATED BY USPA E J23101

1. Make a pre-inoculation of DH5a :: puspA:GFP / / pJ23101 of 10 mL of LB containing 10 µL of 35mg/mL cloramphenicol. Incubate overnight at 37°C under 200 rpm.

2. Add to an Erlenmeyer flask of 500 mL containing 100 mL of LB supplemented with cloramphenicol (ratio of 1 µL/mL of medium), about 5-10 mL of pre-inoculation. Incubate culture at 37°C under 200 rpm until optical density at 600nm of 0.5-0.6 (Near to 1,5-2h)

3. Leave flasks overnight at 4°C without agitation.

4. Centrifuge for 10 min at 4,000 rpm at 4°C.

5. Proceed protein extraction as described in "**PROTOCOL TO PRODUCTION OF [6xHis] - GFP**" (Steps 5 to 8).

6. Pipete 200 µL to a black Elisa plate, and measure in Viktor (PerkinElmer) equipment with Excitation filter of 340nm and emission filter of 500 nm.